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RESEARCH ARTICLE

Factors affecting emergence of *Thaumetopoea pityocampa* in laboratory conditions

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Abstract

In order to explore the factors affecting the emergence of the pine processionary moth (*Thaumetopoea pityocampa*) in laboratory conditions, 1487 larvae, prepupae and pupae were collected in the period February – May 2019 from five pest habitats: Fotinovo and Kandilka Villages (inhabited by a summer phenological form), Sandanski and Klisura Towns (winter phonological form occurs) and Sarnak Village (both forms occur). At the date of collection, the rate of parasitism was very low (0-0.1%). Throughout the study, significant differences between the characteristics of the pupal stage and mortality between both phenological forms were established. The rate of emerged adults varied from 21.4% to 77.6% in the observed habitats. The sex ratio between female and male specimens was almost equal. Only 0.6% of the samples emerged in the second year, after a diapause. At four of the localities (Fotinovo, Kandilka, Sandanski and Sarnak), the rate of infection by entomopathogens was between 6.0% and 20.8%, while the parasitism caused by tachinids was between 0.9% and 3.4%. The flight period of the summer and winter phenological forms of *T. pityocampa* did not coincide with about a month. In the summer form, the flight began in late May and ended to the last decade of June, while in the winter form the flight lasted from early July till the end of August. The duration of the flight period in both forms lasted 30 to 50 days, with an equal number of emerged male and female specimens.

Keywords

Thaumetopoea pityocampa, pupae, emerged adults, phenological forms, Bulgaria

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Introduction

The pine processionary moth, *Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775) (Lepidoptera: Notodontidae) is not only one of the most dangerous defoliating pests of pine stands in the Mediterranean region but also a hazardous allergen for public and animal health. The harmfulness of the pest is determined by its geographic range expansion observed during the recent decades (Battisti et al., 2005, 2006; Mirchev et al., 2011a; Zaemdzhikova et al., 2018). The expansion of affected areas is due to both endogenous (high plasticity and adaptability of the pest) and exogenous drivers. Some factors, as global climate warming (IPCC, 2007; Grunewald et al., 2009; Raev et al., 2010) and increasing the area of coniferous stands (especially pine species) planted on the area of deciduous forests affect the distribution, abundance and population dynamics of *T. pityocampa* (Mirchev et al., 2000).

Several temperature thresholds determine the ecological niche of *T. pityocampa*. Summer temperatures over 32-42 °C (Huchon, Démolin, 1970; Santos et al., 2011; Robinet et al., 2013) are considered as critical for the survival of eggs and young larvae. A wide range of cold temperatures (between -15 and -29 °C) could be lethal for the overwintering larvae (Radchenko, 1927; Russkoff, 1930; Zankov, 1960; Démolin, 1969; Roques et al., 2015). The number of favourable days during the winter (daily temperatures above 9 °C, followed by positive temperatures at night) has a major influence on the successful larval development of the pest (Battisti et al., 2005). The larvae stop feeding at temperatures below 6 °C (Androić, (1956).

Generally, the larvae of the typical (Mediterranean or winter) phenological form of *T. pityocampa* feed and develop during the autumn-winter months and pupate in spring (Douma-Petridou, 1990). An early (summer) phenological form, in which the larvae complete their development before the beginning of the cold months and overwinter in the soil, was established in Portugal (Santos et al., 2013) and Bulgaria (Tschorbadjiew, 1926; Zankov, 1960; Mirchev et al., 2019; Zaemdzhikova et al., 2019). Thus, the larvae of the summer form overcome the factors limiting their development during the winter. In Bulgaria, the geographical distribution of the two phenological forms is relatively well studied (Mirchev et al., 2019; Zaemdzhikova et al., 2019, 2020a).

The diapause is an endogenously regulated dormant state that provides a means for insects to survive seasons of adverse environmental conditions and allows populations to synchronise periods of active development and reproduction with seasons of optimal resource availability (Koštál, 2006). In pupal stage, the diapause of *T. pityocampa* lasts 1-6 years (Strand, 1913; Tsankov, 1959; Zankov, 1960; Démolin, 1969).

The occurrence of two phenological forms of *T. pityocampa* in Bulgaria has encouraged us to study the duration and dynamics of adult emergence, sex ratio and factors causing mortality in the pupal stage.

Material and methods

A total of 1487 larvae, prepupae and pupae of *T. pityocampa* were collected in the period February – May 2019 from five habitats in stands of *Pinus nigra* Arn. in Bulgaria, where the two phenological forms occur (Table 1). The habitats at the land of Fotinovo and Kandilka Villages were inhabited by the summer phenological form (Zaemdzhikova et al., 2019). The winter phenological form is spread in the region of Sandanski (Mirchev et al., 2004) and Klisura (Georgieva et al., 2020a), while in the habitat near the Sarnak Village both phenological forms occur (Mirchev et al., 2019).

Table 1. Main characteristics of the studied localities and pine processionary moth populations

Locality	State Forestry	Phenological form	Collected samples, n	Date of collection	Geographical coordinates		Altitude, m a.s.l.
					Latitude	Longitude	
Fotinovo	Kirkovo	summer	730	06.02.2019	41.378306	25.321639	458
Fotinovo	Kirkovo	summer	390	26.03.2019	41.378306	25.321639	458
Kandilka	Krumovgrad	summer	178	26.03.2019	41.409806	25.581583	464
Sarnak	Krumovgrad	both	117	26.03.2019	41.430694	41.430694	465
Sandanski	Sandandki	winter	42	23.03.2019	41.519750	23.273167	127
Klisura	Rozino	winter	30	22.05.2019	42.702417	24.455333	843

Two collection methods were used: (*i*) digging out pupae from natural pupation sites (Fotinovo, Kandilka, Sarnak and Sandanski) and (*ii*) intercepting mature larvae at the beginning of their procession on tree trunks with collar traps 'Ecopièges' during their way down to the ground (Klisura) and placing them in mesh-capped pots with substrate to stimulate pupation on site.

The samples were transported to the laboratory of entomology at the Forest Research Institute, Sofia. A number of 50-60 samples were placed in sterile moistened sand in plastic boxes with ventilated openings. The sand was moistened weekly. The emergence of adults, parasitoids or entomopathogenic infection was monitored daily. All infected pupae were removed for identification of pathogens. The final observation was carried out in January 2021.

The obtained results were statistically processed with MS Excel 2016.

Results

In the studied habitats of *T. pityocampa*, time of pupation and state of collected pupae varied significantly between both phenological forms (Table 2). Samples collected from Fotinovo and Kandilka indicated that all larvae descended into the soil to pupate

in the period February – March. In samples from the land of Sarnak Village, inhabited by both phenological forms, 86.2% of the recorded specimens were in larval stage.

At the time of the first collection in Fotinovo, no symptoms of pathogenicity were observed in larvae and pupae. At the time of the second collection, in the same habitat, a low number of infected pupae by entomopathogenic fungi were found in Fotinovo (7.9%) and in Kandilka (11.8%). The lowest incidence rate of infection was observed in Sarnak (1.7%). At that time, parasitism by tachinids (Diptera: Tachinidae) was negligibly low: only one specimen was registered in Fotinovo (Table 2).

During the laboratory rearing, symptoms of infection by the entomopathogenic fungi *Beauveria* spp. and parasitism appeared in the samples from the four studied habitats (Fotinovo, Kandilka, Sarnak and Sandanski). The rate of infected by entomopathogens pupae varied between 6.0% and 20.8%, and the parasitism caused by tachinids – between 0.9% and 3.4% (Fig. 1). The absence of infection and parasitism in the samples from Klisura most probably was due to the different method of sample collection, where a contact of larvae with soil was not carried out.

Table 2. Samples characteristics at the time of collection

Collected samples	Locality											
	Fotinovo				Kandilka		Sandanski		Klisura		Sarnak	
Date of collection	06.02.2019		26.03.2019		26.03.2019		23.03.2019		22.05.2019		26.03.2019	
	N	%	n	%	n	%	n	%	n	%	n	%
Pupae	641	87.8	314	80.5	157	88.2	-	-	27	90.0	15	12.1
Pupae with mycelium	-	-	31	7.9	21	11.8	-	-	-	-	3	1.7
Larvae	88	12.1	-	-	-	-	42	100	3	10.0	99	86.2
Tachinid parasitoids	1	0.1	-	-	-	-	-	-	-	-	-	-
Σ	730	100	390	100	178	100	42	100	30	100	117	100

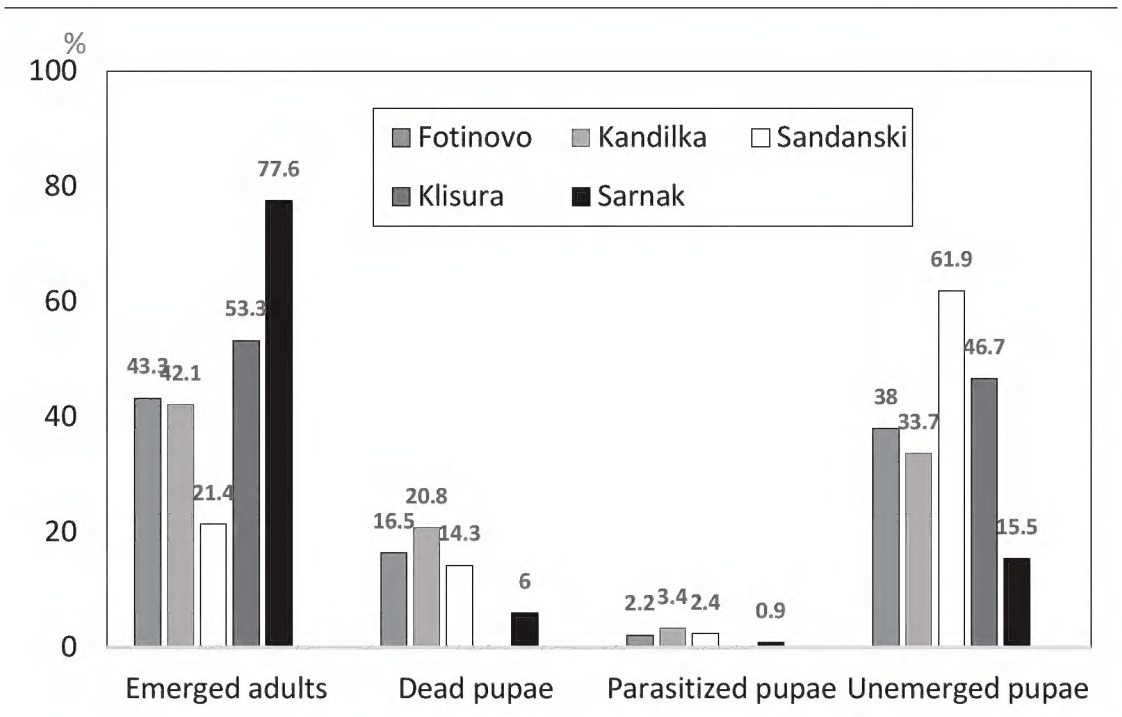


Figure 1. Distribution of samples in studied localities

The relative share of emerged adults varied widely: from 21.4% (Sandanski) to 77.6% (Sarnak) (Fig. 1). Only 0.6% of the adults from Fotinovo appeared in the second year after collection. On the third year after collection, between 15.5% (Sarnak) and 61.2% (Sandanski) of the studied pupae remained unemerged.

The sex ratio between female and male adults emerged from three localities (Fotinovo, Kandilka and Sarnak) was almost equal (Table 3). In Sandanski and Klisura, the sex ratio varied widely, but the number of studied samples was very low (9 and 16 adults, respectively).

Table 3. Sex ratio of emerged adults

Locality	♀		♂		Σ	
	n	%	n	%	N	%
Fotinovo	221	47.6	243	52.4	464	100
Kandilka	37	49.3	38	50.7	75	100
Sandanski	6	66.7	3	33.3	9	100
Klisura	4	25.0	12	75.0	16	100
Sarnak	41	45.6	49	54.4	90	100

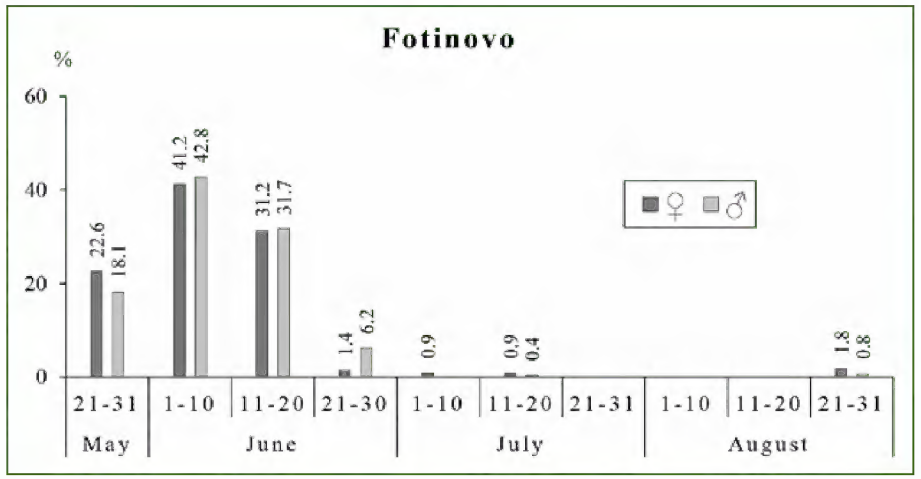
The flight periods of the both phenological forms of *T. pityocampa* were clearly differentiated (Fig. 2). In fact, this is the main objective indicator by which they are defined. The difference between the flight of summer phenological form in Fotinovo and Kandilka and winter form in Sandanski and Klisura, was nearly a month. In the summer form it begun in late May and ended in the last decade of June. The flight of the winter form started in the beginning of July and lasted until the end of August. However, the emergence of adults in the more northern habitat, Klisura started 20 days earlier compared to Sandanski.

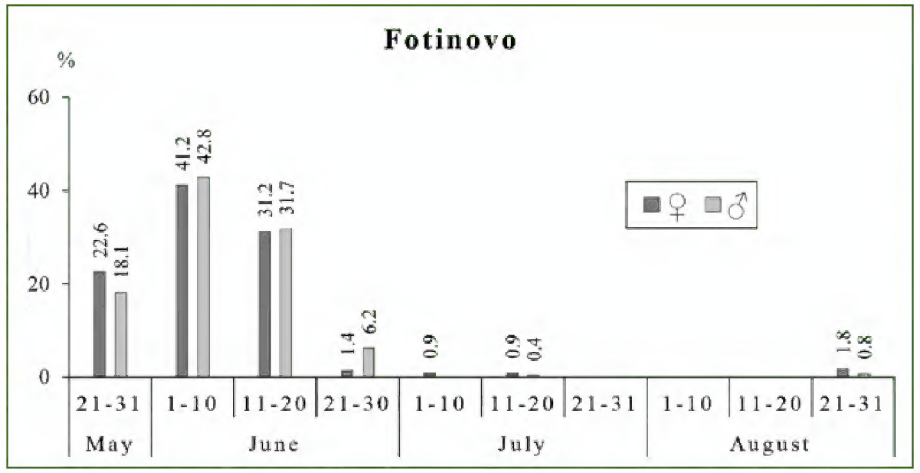
The time and dynamics of *T. pityocampa* flight confirmed the knowledge that in the land of Sarnak both phenological forms occur. The emergence of adults begun in the first half of June and lasted to the first half of August (Fig. 2).

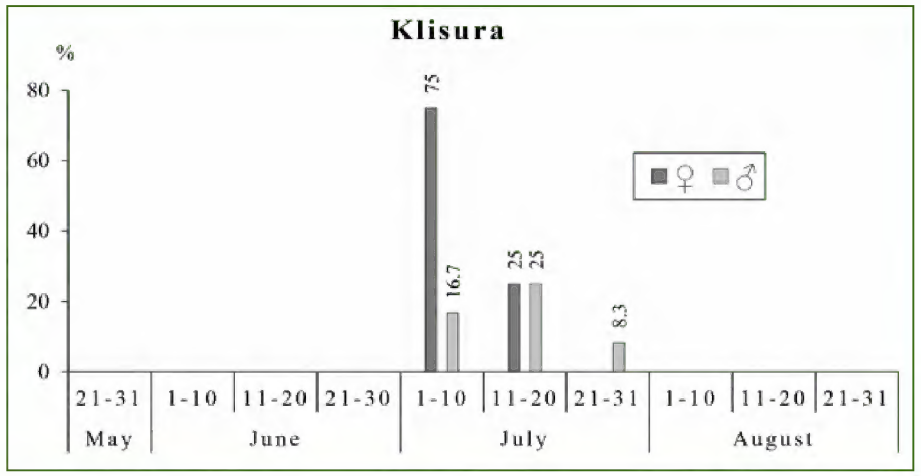
The flight of both phenological forms of *T. pityocampa* lasted 30-50 days. Synchronization of the ratio of newly emerging females and males was observed. Some exceptions were noticed at samples from Sandanski and Klisura, but their number was too low.

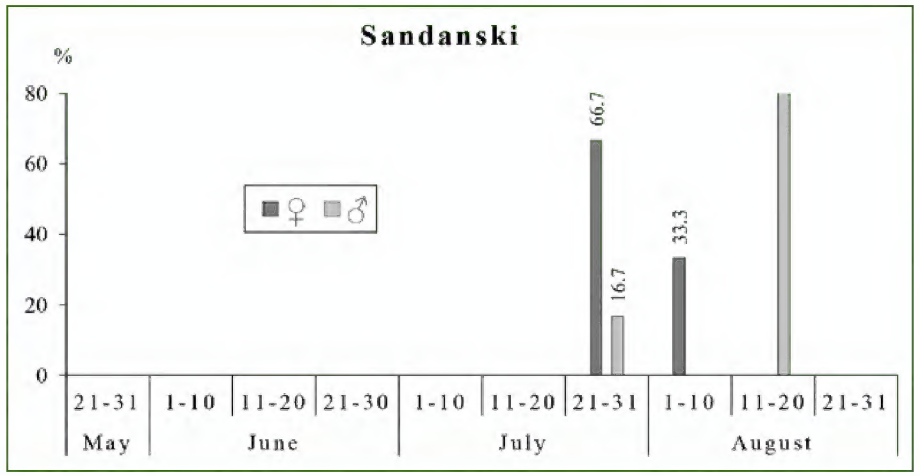
Discussion

In the region of Fotinovo, only the summer phenological form of *Thaumetopoea pityocampa* occurs. In December 2017 and 2019, it was established that all nests were empty and the larvae had descended into the soil (Zaemdzhikova et al., 2019). During the collection in February 2019, 11% of the samples were in larval and pronymphal stage. Tsankov (1959) reported that after descending into the soil in the autumn, the









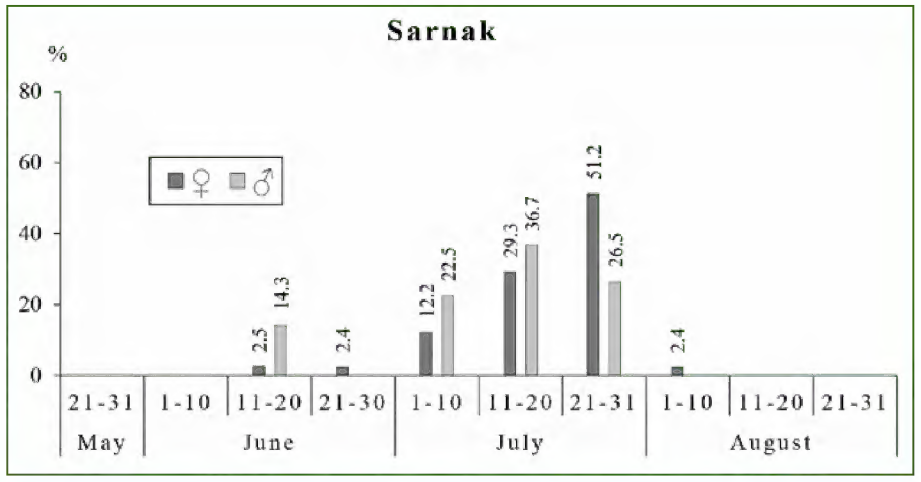


Figure 2. Dynamics of emerged adults

larvae remained in the pronymphal stage and pupated in the spring, showing high resistance to low temperatures. Previously, Russkoff (1930) established these biological characteristics of *T. pityocampa* and observed that single larvae remained to overwinter in the nests. In early spring, a part of them were collected and reared at laboratory conditions. They buried in the sand in late April, pupated in twenty days and emerged in early August.

Out of the samples from the five habitats, only in Fotinovo a low number of adults emerged in second year. This outcome contrasts with the results obtained by other authors. Zankov (1960) found a 3-year diapause of pupae in laboratory conditions, with 56% emergence in the year of collection, 27% – in the next year and 17% remained in diapause. Strand (1913) and Androić (1957) reported that the period of diapause could last two years, and Démolin (1969) – up to six years. According to Androić (1956), the process of diapause could be due to pest physiogenesis or to the influence of the environmental factors.

In this study, tachinid species (Diptera: Tachinidae) parasitized between 0.9% and 3.4% of *T. pityocampa* pupae in four localities of the host. In Bulgaria, Hubenov (1983) reported *Phorocera grandis* (Rondani, 1859) on larvae and *Phryxe vulgaris* (Fallén, 1810) on pupae of the pine processionary moth, and *C. concinnata* on *Thaumetopoea processionea* (Linnaeus, 1758) and *T. solitaria* (Freyer, 1838) (Hubenov, 1985).

In Croatia, Androić (1957) reported several parasitoids on *T. pityiocampa* pupae. Among them, *C. concinnata* was defined as a common parasitoid, and other species, *Ravinia pernix* (Harris, 1780) (syn. *Sarcophaga haemorroidalis* Fallén, 1817) (Diptera: Sarcophagidae), *Coelichneumon rudis* (Fonscolombe, 1847), *Ichneumon coniger* Tischbein, 1876 (Hymenoptera: Ichneumonidae), *Villa hottentotta* (Linnaeus, 1758) (syn. *Anthrax hottentottus* Lundbeck, 1908), *Hemipenthes velutina* (Meigen, 1820) (syn. *Thyridanthrax velutinus* Meigen, 1820) (Diptera: Bombyliidae) and *Conomorium amplum* (Walker, 1835) (syn. *Conomorium eremita* Foerster, 1841) (Hymenoptera: Pteromalidae) were defined as insignificant.

In Fotinovo, *Oecanthus pellucens* (Scopoli, 1763) (Orthoptera: Gryllidae) was registered for the first time as a predator of *T. pityiocampa* larvae (Zaemdzhikova, Doychev, 2020).

Three species of entomopathogenic fungi, *Beauveria pseudobassiana*, *B. varroae* and *Purpureocillium lilacinum*, were identified for first time from naturally infected larvae or pupae of *T. pityocampa* collected in Fotinovo and Kandilka habitats (Barta et al., 2020). The hyperparasitic fungus *Syspastospora parasitica* attacking *B. pseudobassiana* and *B. varroae* was isolated from infected larvae and pupae of pine processionary moths in the region of Fotinovo, Sarnak and Kandilka (Georgieva et al., 2020b).

The survival of wintering *T. pityocampa* larvae in the nests is limited by the extremely low temperatures. The large range of values, from -15 °C (Radchenko, 1927) to -29 °C (Roques et al., 2015), is due to the different combination of the temperature and air humidity. It was established that the mortality of larvae increases significantly

in wet winters (Kafol, 1951) and in habitats with high air humidity (Mirchev et al., 2016).

Russkoff (1930) pointed that the pine processionary moth is separated in two morphologically identical lines (forms). According to the author, the phenology of *T. pityocampa* in Bulgaria differ significantly, compared to knowledge in other countries.

Data showed significant difference on the time of pupation of *T. pityocampa* in the neighbour republics of Former Yugoslavia. For the region of Nikšić (Montenegro), the pest actively feeds in April-May and pupates at the end of May (Radchenko, 1927; Roques et al., 2015). For Croatia, Androić (1957) reports that in some habitats, the larvae descend into the soil earlier. In the laboratory conditions, the period of pupation is too long – from the end of July to the middle of September, but in the natural environment, it is not the same (Androić, 1956). In Yugoslavia, it was observed that the larvae of the pine processionary moth pupated in February, but in areas with cooler climate, it happens much later (Androić, 1978). In Bulgaria, the period of *T. pityocampa* pupate is quite different. However, it is difficult to separate the geographical distribution of the two phenological forms by the indicator climate conditions (if they are cooler or more temperate). The summer phenological form occasionally occurs in zones with harsh cold conditions in winter, where it pupates a few months earlier than the typical Mediterranean form. It was observed that the typical form inhabits the lands of villages in Sofia region characterized by severe winters (Mirchev et al., 2011b, 2016). Recently, *T. pityocampa* was found near Slivnitsa town where the lowest winter temperatures are often measured in Bulgaria (Zaemdzhikova, Doychev, 2019).

In conclusion, it should be noted that the present study increases the knowledge on biological and ecological characteristics of *T. pityocampa* in pupal stage. However, further research is needed to clarify both the factors and mechanisms of genesis and regional distribution of two phenological forms, which is of fundamental and practical importance for pest control.

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